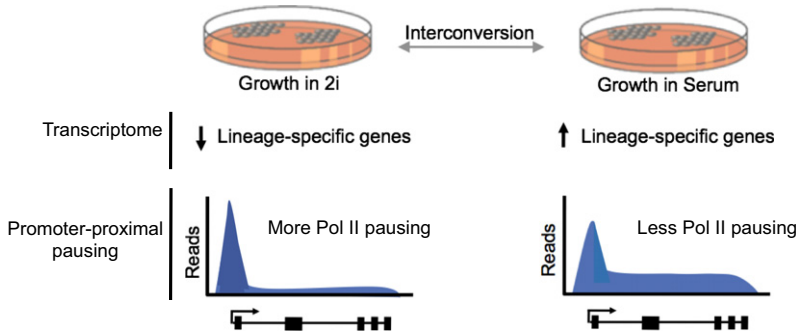

Figure 1. Transcriptional Differences between 2i and Serum-Grown mESCs
Schematic diagram of mESC growth conditions (top), transcriptome (middle), and RNA Polymerase II pausing differences (bottom, Pol II) in mESCs grown in 2i (left) or serum (right). Reads indicate ChIP-Seq reads. Models for lineage-specification genes are shown below the graphs in black, and start sites are indicated by an arrow. The transcriptional state of mESCs grown in either condition is readily interconverted by changing the growth media.

and maintaining the ground state of naive pluripotent ESCs.

Marks et al. (2012) found that the transcriptional and chromatin states of mESCs were interconvertable when cells were switched from one growth condition to the other. This observation indicates that whatever molecular memory mESCs have of one state is rapidly lost when exposed to the other condition.

Cultured human ESCs have features that indicate that they are more developmentally advanced than mESCs, and there is considerable interest in isolating human pluripotent stem cells in a more naive state (reviewed in De Los Angeles et al., 2012). This is challenging because the simple application of 2i conditions to cells isolated from preimplantation human embryos or from reprogramming has thus far failed to produce naive human ESCs/iPSCs. It seems likely that naive human ESCs will eventually be isolated, and it will then be interesting to determine if enhanced Pol II pausing and other features of the transcriptional and chromatin landscape described by Marks et al. (2012) are also found in these human cells.

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Autoimmune T Cells Lured to a FASL Web of Death by MSCs

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Achieving immune tolerance through cell transplantation is a promising approach for treating autoimmune disease. In this issue of *Cell Stem Cell*, Akiyama et al. (2012) demonstrate that human and mouse mesenchymal stem cells can induce immune suppression by attracting and killing autoreactive T cells, which stimulates TGFb production by macrophages and generates regulatory T cells.

Mesenchymal stem cells/marrow stromal cells (MSCs) are perhaps the cell type most commonly investigated for tissue repair therapies. However, MSCs appear capable of doing much more than simply facilitating tissue repair. These interesting cells, derived most often from the bone marrow or fat, can serve as "paramedics" to help heal tissue through local and

systemic secretion of a diverse array of cytokines. To date, MSCs from bone marrow and adipose tissue have been extensively tested and proven effective in preclinical animal models of many injuries and disorders and are actively being investigated clinically for many disease states (reviewed in Meyerrose et al., 2010). Following systemic delivery, MSCs are capable of extensive migration, in particular to areas of hypoxia, inflammation, or other tissue injury, and release trophic factors that hasten endogenous repair (Caplan and Correa, 2011). These secreted bioactive products can enhance angiogenesis, inhibit fibrosis and apoptosis, and stimulate recruitment, retention, proliferation, and differentiation of tissue-residing stem cells. Importantly, MSCs appear to be potently immunosuppressive, as they can be infused without tissue matching and are able to shield themselves from the host immune attack (Tolar et al., 2010; English and Mahon, 2011). The ability to be transplanted without tissue matching has allowed large multicenter trials to be conducted with direct comparison of the same batches of MSCs without adverse events or overt rejection reactions. Human MSCs are currently being tested in clinical trials for numerous applications including, but not limited to, myocardial infarction, stroke, knee injury, Crohn's disease, critical limb ischemia, neurodegenerative disorders, kidney disease, graft-versus-host disease (GVHD), and various autoimmune disorders (Caplan and Correa, 2011; Tolar et al., 2010). It is their immunosuppressive capability that attracts much attention because it not only allows for a greater donor pool to choose from but also may allow their use in transplantation and promoting donor engraftment. However, the mechanisms by which MSCs modulate immune function have been difficult to decipher. Nonetheless, these cells would seem uniquely suited to be used clinically in autoimmune states due to their immunomodulatory capabilities as well as their regenerative abilities.

In the current issue of *Cell Stem Cell*, Akiyama et al. (2012) report a fascinating new mechanism by which MSCs exert their immunosuppressive effects through multiple paracrine interactions and cell-cell contacts. MSCs appear to express functional FAS ligand (FASL), more commonly associated as a potent effector arm of cytotoxic T cells, and after adoptive transfer were shown to be capable of directly mediating apoptosis of host T cells that express FAS. This effect was specific for the FAS-FASL interaction because FASL-deficient MSCs failed to promote T cell apoptosis. Another novel (and complex) twist in this scenario is that MSCs first lure autoreactive T cells via the chemokine MCP-1, and this chemokine is induced by triggering of FAS on the MSC, creating a complex dance in which both ends of FAS-FASL interactions act on the MSC. This pathway of inducing T cell attraction represents a novel means to increase the likelihood that the host T cells will come into contact with the MSC. Death of the effector T cells via FASL is only part of the story because their demise also causes the production of TGFb by macrophages, which engulf the dying cells, and then leads to generation of potently immunosuppressive regulatory T cells (Tregs), which further suppress immune function. Thus, MSCs are capable of potent immunosuppression via a multipronged approach consisting of both direct and indirect effects.

What makes the study by Akiyama so informative is that, although there have been significant differences between mouse and human MSCs with regard to the pathways used in immune suppression (Ren et al., 2009), the study shows similar effects and end results of MSCs in both a mouse model of colitis and patients with systemic sclerosis. In both cases, allogeneic MSC transfer resulted in T cell loss, increase in Treg numbers, and significant resolution of disease pathology. This study raises tantalizing possibilities with regards to application of this approach for other autoimmune disorders and organ transplantation. MSCs appear to exert their immunosuppressive effects by acting as what immunologists call "veto cells" in which recognition by the host effector cells is needed to trigger a reaction that leads to their death. Here, reactive T cells come into contact with a veto cell (in this case an MSC) and are killed as a result. The use of the veto phenomena has been under examination using donor T and NK cells as a means to promote deletion of donor-reactive host T cells, thus resulting in greater donor engraftment and perhaps permanent immune tolerance in organ transplantation (Reich-Zeliger et al., 2007). MSCs take it another step further by providing the honey (MCP-1) to attract the flies (host T cells). The study also raises important questions with regards to previous reports where the immunosuppressive capabilities of MSCs have been less clear. If these cells have such weapons in their arsenal, reports of allogeneic MSCs being immunogeneic and rejected by recipient mice (Nauta et al., 2006), and clinical trials using these cells in GVHD (Tolar et al., 2010; Le Blanc et al., 2008) yielding equivocal results with regard to improvement of pathology, show that much still needs to be delineated with regards to clinical exploitation of MSCs for immunosuppression. It will also be important to ascertain the extent of the immunosuppression that these cells can exert using much more quantitative assays for in vivo immune responses. Other questions also are raised as to how the triggering of FAS on the MSC results in MCP-1 production and not death. Clearly, there must be thresholds in the spectrum of responses, and these must be delineated to determine and optimize their full potential.

The study also raises intriguing questions as to why such immunosuppressive properties exist in these cells. It is perhaps surprising that FASLis expressed on a nonimmune cell type and even more so that the cell has evolved to express chemoattractants that draw T cells to them. Maybe nature intended that these cells have the ability to roam to sites of organ damage to not only promote tissue repair but to also immunosuppress at their own discretion and prevent potential autoimmune triggering that may occur at the site of injury. Regardless of why, these are properties that we are more than happy to take advantage of in the clinic for the treatment of various disease states.

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Early Embryos Reprogram DNA Methylation in Two Steps

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While DNA cytosine methylation is relatively stable in somatic tissues, it is highly dynamic during preimplantation development. A recent study in *Nature* by Meissner and colleagues (Smith et al., 2012) now reveals dramatic shifts in DNA methylation during the earliest stages of mouse embryogenesis at genome scale and base resolution.

DNA methylation at the 5-position of cytosine (5mC) in mammals largely occurs at CpG dinucleotides and is required for normal embryogenesis. A global DNA methylation landscape is first established by de novo DNA methyltransferases Dnmt3a and Dnmt3b in the inner cell mass (ICM) of blastocysts and is stably inherited in somatic tissues through the action of maintenance methylation machineries. DNA methylation in somatic cells generally displays a bimodal distribution, in which the majority of CpG sites are methylated and unmethylated CpGs are primarily found in clusters termed CpG islands (CGIs) that are frequently associated with gene promoters (Deaton and Bird, 2011). It is broadly accepted that DNA methylation at gene promoters and other regulatory sequences such as enhancers inversely correlates with transcription and may facilitate lineage restriction during development.

While the methylation pattern is stably maintained in somatic tissues, mammalian preimplantation development is accompanied by a wave of genome-wide demethylation and remethylation. Early studies using immunofluorescence staining and locus-specific bisulphite sequencing have indicated that DNA methylation in the paternal genome is rapidly removed via a replication-independent process a few hours after fertilization, while the maternal DNA methylation level is gradually reduced in a replication-dependent manner with the lowest level occurring at the blastocyst stage (Mayer et al., 2000). Recent studies have revealed that loss of 5mC in the paternal genome in the zygote is primarily initiated by Tet3 (Gu et al., 2011; Wossidlo et al., 2011), a member of the Ten-eleven translocation (Tet) family of DNA dioxygenases capable of converting 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) through iterative oxidation (Wu and Zhang, 2011). High-resolution metaphase chromosome immunostaining of various stages of preimplantation embryos using antibodies specific for 5mC, 5hmC, 5fC, and 5caC suggested that the bulk 5mC in the paternal genome is first oxidized to 5hmC/5fC/5caC in zygotes, followed by replication-dependent dilution of 5hmC/5fC/5caC during preimplantation development (Inoue et al., 2011; Inoue and Zhang, 2011) (Figure 1A). Although these studies have revealed a global picture of how 5mC is lost during preimplantation development, it lacks the resolution for us to tell exactly which part of the genome is subject to or protected from this wave of DNA demethylation. Bisulfite sequencing has been the method of choice to generate single-nucleotide resolution maps of DNA methylation, and genome-wide bisulphite sequencing of somatic and cancer genomes continues to shed light on genomic distribution and regulatory function of DNA methylation in tissue-specific gene expression and tumorigenesis. However, similar analysis for preimplantation embryos has been difficult due to limitations on the number of embryos available.

In a recent issue of *Nature*, Meissner and colleagues have successfully overcome this technical hurdle by using the reduced representation bisulphite sequencing (RRBS) technique and have generated base-resolution and genome-scale maps